FORM PTO-1390 (REV 12-29-99)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 41618

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/831623

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/24018	12 November 1999	13 November 1998

TITLE OF INVENTION
SELECTION SYSTEM FOR GENERATING EFFICIENT PACKAGING CELLS FOR LENTIVIRAL VECTORS

APPLICANT(S) FOR DO/EO/US
Ryan McGuinness & Luigi Naldini

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☑ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☑ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. ☑ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☑ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2))
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☑ Other items or information:

3 sheets of formal drawings

First Page of the Published Application

Form PCT/IB/308

International Search Report

Form PCT/IPEA/402

International Preliminary Examination Report

JC18 Rec'd PCT/PTO 11 MAY 2001

U.S. APPLICATION NO (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/831623	PCT/US00/15588	41618

17. ☑ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$970.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$840.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$690.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$670.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	**$96.00**	

ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 690 00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	2 - 20 =	0	X **$18.00**	$	
Independent claims	2 - 3 =	0	X **$78.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$260.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$ 690.00	
Reduction of 1/2 for filing by small entity, if applicable. Small Entity Status applies				$ 345.00	
SUBTOTAL =				$ 345 00	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 345 00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3 28, 3 31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 345.00	
				Amount to be refunded:	$
				charged:	$

a. ☑ A check in the amount of $ 345.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No 0 in the amount of $_____ to cover the above fees
A duplicate copy of this sheet is enclosed.

c. ☑ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 18-2220. A duplicate copy of this sheet is enclosed.

The Declaration/Power of Attorney and Assignment will be filed shortly.

Priority is claimed from US 60/108,169 filed 13 November 1998.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

Roylance, Abrams, Berdo & Goodman, L.L.P.

1300 19th Street, N.W., Suite 600

Washington, D.C. 20036

(202) 659-9076

SIGNATURE

Dean H. Nakamura

NAME

33,981

REGISTRATION NUMBER

Initial Information Data Sheet

Inventor Information	
Inventor One Given Name::	Ryan
Family Name::	McGuiness
Postal Address Line One::	4112 Norton Avenue
City::	Oakland
State or Province::	California CA
Postal or Zip Code::	94602
Citizenship Country::	USA
Inventor Two Given Name::	Luigi
Family Name::	Naldini
Postal Address Line One::	Corso Lepanto 12
City::	Torino
State or Province::	Italy ITX
Postal or Zip Code::	10134
Citizenship Country::	Italy
Correspondence Information	
Name Line One::	Dean H. Nakamura
Name Line Two::	Roylance Abrams Berdo & Goodman
Address Line One::	1300 19th Street, N.W.
City::	Washington
State or Province::	DC
Postal or Zip Code::	20036
Telephone::	(202) 659-9076
Fax::	(202) 659-9344
Electronic Mail::	dnakamura@roylance.com
Application Information	
Title Line One::	Selection System for Generating Efficient
Title Line Two::	Packaging Cells for Lentiviral Vectors
Total Drawing Sheets::	3
Formal Drawings?::	Yes
Application Type::	Utility
Docket Number::	41618
Representative Information	
Registration Number::	33,981

Selection System For Generating Efficient Packaging Cells For Lentiviral Vectors

BACKGROUND OF THE INVENTION

Generation of efficient packaging cell lines for lentiviral vectors is hampered by the cytotoxicity of some of the products of the gag and pol genes. Thus, it is desirable to have inducible expression of gag and pol so that optimal clones that will express gag and pol at high levels when needed can be selected in the absence of gag/pol expression.

SUMMARY OF THE INVENTION

A method for selecting cells which express gag and pol and thus are useful as packaging cells is obtained by linking a selectable marker to the gag/pol expression cassette of a packaging vector in such a way that the marker is expressed by the same promoter which controls expression of the gag/pol genes although expression of the gag/pol genes is suppressed. Efficient expression of the marker predicts efficient expression of the gag/pol genes on induction.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a recombinant vector which exemplifies the instant invention. The gag/pol sequences are flanked by splice donor and splice acceptor sites. Also contained within the splice donor and splice acceptor sites is an RRE (Rev responsive element).

Figure 2 depicts the mechanism by which a vector containing an RRE would provide inducible expression of only the marker gene, in the case of the exemplary recombinant vector of Figure 1, the marker is CD4. In the absence of Rev, splicing occurs between the splice donor and splice acceptor sites thereby eliminating the gag/pol sequence.

Only CD4 is expressed. When Rev is present, splicing does not occur and the gag/pol genes are expressed.

Figure 3 is a graph depicting the amounts of p24, a product of the gag gene, in culture medium when cells containing a vector of the instant invention are propagated in the presence or absence of Rev. Two different vectors were used, MDH spl CD4 and MD L g/p RRE. In both vectors, the gag/pol genes are framed by splice donor and splice acceptor sites and thus p24 is expressed when Rev is present in the culture.

DETAILED DESCRIPTION OF THE INVENTION

The invention takes advantage of the splicing control mechanisms of HIV and other lentiviruses which regulate expression of the late viral genes, gag/pol and env, by means of a cis acting RNA element, RRE, and a trans acting regulatory protein, Rev. By the strategic placement of splice control elements, a switch in a gag/pol expression construct allows expression of a downstream selectable marker gene in the basal state and of the upstream gag/pol genes only on induction. As both genes are driven by the same constitutive promoter, operation of the switch allows for gag/pol induction to an expression level related to that of the selectable marker.

Three features operate the switch: 1) the gag/pol genes are contained within a splice donor site and one or more splice acceptor sites, wherein the sequences of the acceptor sites do not match the optimal consensus splice acceptor sequence (Lewin, "Genes", John Wiley & Sons, NY) upstream of the marker gene; 2) the gag/pol genes contain sequences which antagonize the expression of gag/pol (Schneider et al., J. Virol. 71:4892-4903, 1997; Schwartz et al., J. Virol. 66:7176-7182, 1992); and 3) the gag/pol genes are linked in cis to the RRE element as well as being separated from the Rev coding sequence.

A promoter which controls the expression of both gag/pol and the marker gene is situated operably thereto, generally upstream from the gag/pol sequences.

The RRE/Rev regulatory system is found in lentiviruses and thus, that of HIV-1 or any other lentivirus can be used. Also, any other trans complementing regulatory system which results in selective splicing which would control the expression of gag/pol as described herein can be used in the practice of the instant invention.

The first two features combine to suppress gag/pol expression in the basal state. The third feature allows for Rev-dependent stimulation, i.e., induction, of the export of unspliced RNA and consequent expression of the gag/pol genes.

Regarding the splice sites, a combination of an efficient splice donor site, such as that of the 5' major splice donor of HIV, and one or more splice acceptor sites, wherein the splice acceptor sites do not match exactly the optimal consensus, are used. Therefore, the splice acceptors of interest are those an artisan would recognize as not being that efficient, strong or good. Nevertheless, the splice acceptor sites are operable, albeit at a suboptimal rate of efficiency. The suboptimal splice sites appear to allow for more efficient expression from unspliced transcripts by the Rev-RRE system. An example of such a suboptimal splice acceptor site is that of the third exon of the HIV-1 tat and rev genes.

Non-lentivirus splice donor and splice acceptor sites also can be used in the practice of the instant invention so long as the splicing, and hence expression, of the gag/pol genes is controlled by the presence of a trans acting factor, such as Rev.

The intrinsic instability of the lentiviral gag/pol coding sequences, and particularly the sequences contained in the intron, counteracts expression in the basal state from unspliced transcripts that may accumulate due to the suboptimal nature of the splice sites. Any sequence which is known to be associated with the instability of transcripts can be used in the

practice of the instant invention. Instability sequences, however, such as those identified described in Schneider et al. and Schwartz et al., supra, in the gag/pol sequence, may not be strictly required for the operation of the switch.

Any of a number of possible selectable markers can be used. Markers which are readily detectable are desirable. For example, the marker may be a cell surface molecule, which is antigenic, such as a CD molecule or lymphocyte antigen, or a light-emitting molecule, such as green fluorescent protein. An artisan is free to select a selectable marker of interest from those known in the art.

The methods for cloning the various elements of the instant invention into a vector of interest are known in the art.

As a means of introducing yet another level of regulation, expression of the trans acting splice regulatory elements, in the case of HIV-1, Rev, can be inducible as well. In the presence of a separate inducible Rev expression construct, the expression of the gag/pol genes becomes inducible. For example, expression of Rev can be inducible using the tetracycline dependent regulatory system of Ory et al. (Proc. Natl. Acad. Sci. 93:11400-11406, 1996) wherein Rev is subcloned adjacent to a tet operator. In the presence of tet, Rev is not expressed. However, when tet is withdrawn from the medium, Rev expression occurs.

Other known regulatory elements can be used as known in the art. Thus, a suitable and known promoter can be place operably in the construct to regulate expression of the gag/pol and marker genes. Other regulatory elements, such as a polyadenylation site can be used as desired.

Moreover, various modifications can be made to any one element included in the vectors of interest to remove undesirable activities or to enhance desired activities. The

artisan can rely on the known activities of the elements contemplated and can practice known techniques to effect the desired changes, for example, deletion of sequences by selective subcloning, inactivation of a gene by site directed mutagenesis and so on.

An advantage of the instant invention is selection of optimal packaging clones for vectors, such as lentiviral-derived, and particularly, HIV-derived, vectors. Using a surface marker for the linked selection, a population of stable, high-level expressors can be sorted on transfection of the constructs, and subsequently as often as needed to maintain performance. In previously described linked-selection systems, expression of the marker gene is coupled to the expression of the desired gene and cannot be operated in the reverse direction.

The instant method also can be used to select packaging clones for lentiviral vectors other than HIV-1, either using the HIV-1 Rev-RRE system, or homologous elements of other lentivirus, so long as the homologous regulatory elements functionally operate equivalently to yield selectable splicing of the gag/pol sequence in the presence of an inducer molecule located in trans to the coding sequences of interest.

The invention now will be exemplifed in the following non-limiting examples.

EXAMPLES

A packaging vector, pMDH L g/p RRE Spl CD4 (Figure 1) was constructed to include the following: immediate/early enhancer/promoter of the human cytomegalovirus (CMV); HIV major 5' splice donor; HIV gag/pol coding regions with optimised translation initiation sequence fitting the Kozak consensus sequence (Dull et al. J. Virol. 72:8463-8471, 1998); HIV RRE element; HIV splice acceptor sites from the 3rd exon of tat and rev; human CD4 coding region; and rat insulin poly-adenylation sequence.

The lentiviral packaging vector pMDH L g/p RRE Spl CD4 allows for selection of high level expression of the surface marker CD4 with very low expression of the HIV-1 gag/pol genes. Due to the linkage of the CD4 marker to the gag/pol genes, high expression of CD4 correlates with high inducible expression of gag/pol. In the absence of HIV Rev, splicing of the gag/pol sequences between the HIV splice donor and acceptors yield efficient expression of CD4 without appreciable expression of gag and pol (Figure 2A). In the presence of Rev, the RRE-mediated export of unspliced gag/pol message allows expression of the gag pol proteins (Figure 2B).

The pMDH L g/p RRE Spl CD4 plasmid was transfected into 293T (Dull et al., supra) with or without a Rev expression plasmid (Dull et al., supra) and with a combination of other plasmids required to generate lentiviral vector delivery of a selectable marker, green fluorescent protein (GFP).

About $4x10^6$ 293T cells were plated per 10 cm dish the night prior to transfection. $CaPO_4$ co-transfection of the following plasmids was performed: pMDH L g/p RRE Spl CD4, 7μg (HIV-derived gag/pol expression plasmid); pRSV Rev, 2.5μg; pCMV tat, 1μg; pMD VSVG env, 3.5μg; and pRRLhPGKGFPSIN-18, 10μg (a self-inactivating HIV-derived transfer vector carrying a green fluorescent protein coding sequence linked to a PGK promoter). Identical transfections also were performed without the pRSV Rev plasmid, and with the parental packaging vector pMD L g/p RRE in place of pMDH L g/p RRE Spl CD4. Twenty hours after transfection, fresh medium was added and 24 hours later, conditioned medium was harvested for measuring the content of the HIV gag product, p24, by immunocapture (Dupont) and for assaying transduction. The transfected cells were harvested, incubated with phycoerythrin-labelled anti-CD4 antibodies and analyzed by FACS for phycoerythrin and GFP fluorescence.

The transfectants were analyzed for expression of both CD4 and GFP, with and without HIV Rev. In both cases the vast majority of cells were doubly positive for CD4 and GFP. As expected, the average expression level of CD4 was higher in cells not expressing Rev. Expression of basal levels CD4 in the presence of Rev is due to the fact that Rev does not prevent completely the splicing of RRE-containing transcripts.

Similar transfections also were performed with the parental packaging vector pMD L g/p RRE in place of pMDH L g/p RRE Spl CD4. The vector pMD L g/p RRE expresses gag/pol of HIV in a Rev-dependent manner downstream of a constitutively spliced intron derived from the ß-globin gene. Cells co-transfected with the pMD L g/p RRE packaging vector and Rev expressed gag/pol whereas in the absence of Rev, no gag/pol was detected.

Expression of the gag/pol genes in the transfected 293T cells was analyzed by measuring the content of the gag gene product, p24, in the conditioned medium by immunocapture (DuPont). Figure 3 shows the p24 concentration in the conditioned media of cells transfected with both packaging vectors in the presence and absence of HIV Rev. The Rev dependence of gag/pol expression for both plasmid is evident. The plasmid which contains the CD4 coding sequence expresses a very high level of p24 protein in the presence of Rev, similar to that obtained with the control plasmid.

Production of functional vector was analyzed by using the 293T conditioned medium to transduce the GFP gene into HeLa cells. HeLa cells were exposed to 10 µl of medium conditioned by cells transfected with the pMDH L g/p RRE Spl CD4 packaging vector or the pMD L g/p RRE packaging vector in the presence (a) and absence (b) of HIV Rev. Transduction experiments were carried out by plating $5x10^4$ cells/well in 6-well plates the night prior to infection. The next day, frozen 293T conditioned medium was thawed and diluted 1:10, 1:100, 1:1000, and 1 ml of each dilution was used to infect the cells. Twenty

hours after infection, fresh medium was added and 24 hours later, cells were analyzed by FACS for GFP expression.

The Rev-dependence of the transduction was evident for both plasmids. Only when Rev is expressed in vector producer cells do the target HeLa cells express GFP. Moreover, the infectivity (transducing units/ng p24) of vector produced by either plasmid is similar, indicating that the CD4-linked plasmid operates as efficiently as the control plasmid.

We claim:

1. A nucleic acid construct comprising in operable linkage in the 5' to 3' direction;

(1) a promoter;

(2) a splice donor site;

(3) a gag/pol coding sequence;

(4) a Rev responsive element or functional equivalent thereof;

(5) a splice acceptor site; and

(6) a selectable marker coding sequence.

2. A composition comprising:

(a) a first expression cassette comprising in operable linkage in the 5' to 3' direction:

(1) a promoter;

(2) a splice donor site;

(3) a gag/pol coding sequence;

(4) a Rev responsive element or functional equivalent thereof;

(5) a splice acceptor site; and

(6) a selectable marker coding sequence; and

(b) a second expression cassette comprising in operable linkage in the 5' to 3' direction:

(1) a promoter; and

(2) a nucleic acid encoding a factor which binds to element (4) of said first expression cassette, which on such binding regulates splicing at said sites (2) and (5) of said first expression cassette when an mRNA is transcribed from said first expression cassette.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

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A1

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(71) Applicant *(for all designated States except US)*: CELL GENESYS, INC. [US/US]; 342 Lakeside Drive, Foster City, CA 94404 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MCGUINNESS, Ryan [US/US]; Cell Genesys, Inc., 342 Lakeside Drive, Foster City, CA 94404 (US). NALDINI, Luigi [IT/IT]; Cell Genesys, Inc., 342 Lakeside Drive, Foster City, CA 94404 (US).

(74) Agents: NAKAMURA, Dean, H. et al.; Sughrue, Mion, Zinn, MacPeak & Seas, PLLC, Suite 800, 2100 Pennsylvania Avenue, Washington, DC 20037-3213 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

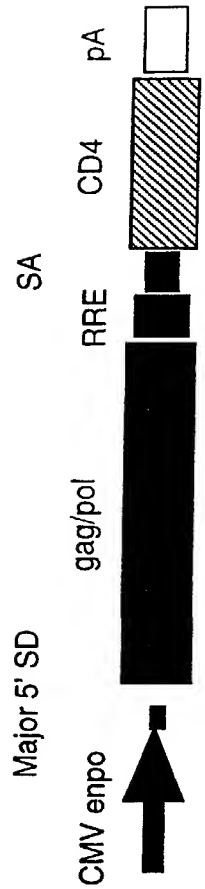
Published
With international search report.

(54) Title: SELECTION SYSTEM FOR GENERATING EFFICIENT PACKAGING CELLS FOR LENTIVIRAL VECTORS

(57) Abstract

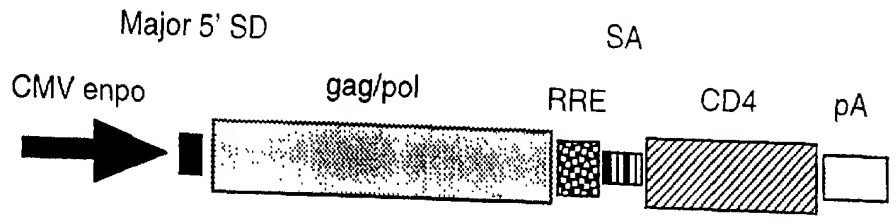
A method for selecting packaging cells that express high levels of gag/pol is provided.

Figure 1 pMDH L g/p RRE Spl CD4

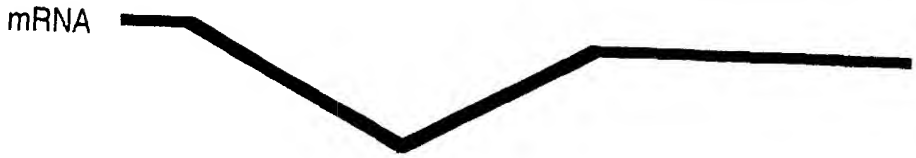


CMV enpo: human CMV immediate early enhancer and promoter
Major 5' SD: HIV 5' splice donor
gag/pol: HIV gag and pol coding regions
RRE: HIV Rev Responsive Element
SA: HIV tat 3rd exon splice acceptor region
CD4: Human CD4 coding region
pA: Rat insulin poly adenylation site

Figure 2



A. Absence of Rev permits splicing to occur between the splice donor and acceptors, thus eliminating gag/pol sequences and expression of their gene products. CD4 is efficiently expressed from this messenger RNA.



B. Presence of Rev suppresses splicing resulting in inclusion of gag/pol sequences and expression of their gene products.

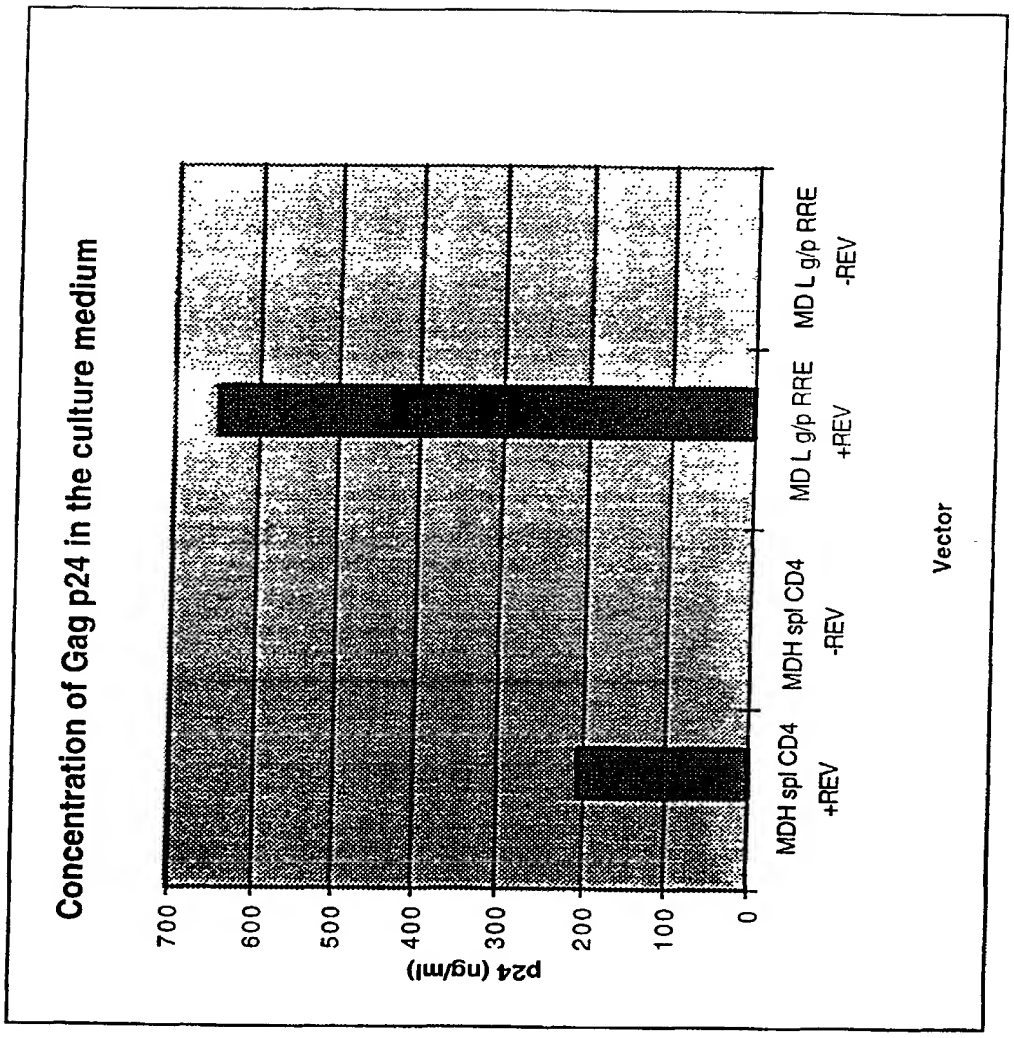

Concentration of Gag p24 in the culture medium
p24 (ng/ml)
0
100
200
300
400
500
600
700
MDH spl CD4 +REV
MDH spl CD4 -REV
MD L g/p RRE +REV
MD L g/p RRE -REV
Vector

Figure 3

PTO/SB/01A (10-00)
Approved for use through 10/31/2002 OMB 0651-0032
U S Patent and Trademark Office, U S DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

As the below named inventor(s), I/we declare that:

This declaration is directed to:

☐ The attached application, or

☑ Application No. 09/831,623, filed on 11 May 2001,

☐ as amended on _____(if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/ we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including material information which became available between the filing date of the prior application and the National or PCT International filing date of the continuation-in-part application, if applicable; and

All statements made herein of my/own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: Ryan McGuinness

Signature: Ryan McGuinness Citizen of: USA

Inventor two: Luigi Naldini

Signature: _____ Citizen of: _____

Inventor three: _____

Signature: _____ Citizen of: _____

Inventor four: _____

Signature: _____ Citizen of: _____

☐ Additional inventors are being named on _____ additional form(s) attached hereto.

Burden Hour Statement This collection of information is required by 35 U S C. 115 and 37 CFR 1.63. The information is used by the public to file (and the PTO to process) an application. Confidentiality is governed by 35 U S.C. 122 and 37 CFR 1.14. This form is estimated to take 1 minute to complete. This time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U S Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231

PTO/SB/01A (10-00)
Approved for use through 10/31/2002. OMB 0651-0032
U S Patent and Trademark Office, U S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

As the below named inventor(s), I/we declare that:

This declaration is directed to:

☐ The attached application, or

☑ Application No. 09/831,623, filed on 11 May 2001,

☐ as amended on _____(if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/ we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including material information which became available between the filing date of the prior application and the National or PCT International filing date of the continuation-in-part application, if applicable; and

All statements made herein of my/own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: Ryan McGuinness

Signature: _____ Citizen of: _____

Inventor two: Luigi Naldini

Signature: [signature] Citizen of: ITALY

Inventor three: _____

Signature: _____ Citizen of: _____

Inventor four: _____

Signature: _____ Citizen of: _____

☐ Additional inventors are being named on _____additional form(s) attached hereto.

Burden Hour Statement: This collection of information is required by 35 U S C. 115 and 37 CFR 1.63. The information is used by the public to file (and the PTO to process) an application Confidentiality is governed by 35 U S.C 122 and 37 CFR 1.14. This form is estimated to take 1 minute to complete. This time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Ryan McGuinness et al.	Assignee:	Cell Genesys, Inc.
Serial No.:	09/831,623	Examiner	Not yet assigned
Filed:	May 11, 2001	Group Art Unit:	Not yet assigned
		Docket:	G&C 131.13-US-WO

Title: SELECTION SYSTEM FOR GENERATING EFFICIENT PACKAGING CELLS FOR LENTIVIRAL VECTORS

CERTIFICATE OF MAILING OR TRANSMISSION UNDER 37 CFR 1 8

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner for Patents, Washington, D C 20231 on 6 Dec 2001

By _____
Name: Karen S. Canady

REVOCATION AND POWER OF ATTORNEY

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please revoke the existing Powers of Attorney, if any, and appoint the following attorneys and/or patent agents to prosecute this application and to transact all business in the U.S. Patent and Trademark Office in connection therewith:

George H. Gates	Registration No. 33,500
Victor G. Cooper	Registration No. 39,641
Karen S. Canady	Registration No. 39,927
William J. Wood	Registration No. 42,236
Jason S. Feldmar	Registration No. 39,187
Bradley K. Lortz	Registration No. 45,472
Kenneth M. Goldman	Registration No. 34,174
Linda R. Judge	Registration No. 42,702

Please direct all correspondence in this application to Karen S. Canady at:

CUSTOMER NUMBER 22462.
Gates & Cooper LLP
Howard Hughes Center
6701 Center Drive West, Suite 1050
Los Angeles, California 90045
Telephone: (310) 641-8797

Cell Genesys, Inc., as assignee, declares that 100% ownership is established for the above-referenced invention, and patent application. A Certificate under 37 C.F.R. 3.73(b) is enclosed.

Respectfully submitted,

Cell Genesys, Inc.

Dated: 13 November 2001

By: Kenneth M Goldman

Kenneth M. Goldman
Director Intellectual Property